a mix survived differently in individual animals and in general, the culture combination resulted in good survival and persistence, as previously observed by Pedersen *et al.* (1992). Many studies have reported reductions in intestinal coliform and *Enterobacteriaceae* due to probiotic administration; however, some have seen no effects (Simon *et al.,* 2003). In the present study most of the cultures resulted in reductions in faecal *Enterobacteriaceae*; in fact, reductions of up to 98 % were observed. However, except for day 15, these reductions were not statistically significant, probably due to the variation in counts between individual animals, a common observation in probiotic animal trials. This inconsistency may be explained by individual variations in response to probiotics due to the complexity of the intestine (Simon *et al.,* 2003). Future experiments using larger treatment groups and deliberate *Salmonella* infection should provide further information on the pathogen-lowering ability of these cultures.

Conclusions: Pig-derived potentially probiotic cultures with anti-*Salmonella* activity can be effectively delivered to the porcine intestine by oral administration, either individually or as a strain combination. However, it was evident that certain cultures survived at higher levels, persisted for longer in the caecum post-administration and were more effective in reducing pathogenic indicator species, highlighting the advantages of using combination probiotics in pigs. We conclude that, although further characterisation of efficacy is necessary, the findings provide a basis to further explore the potential of these porcine isolates as microbial feed additives (most likely administered as a mixture) for *Salmonella* reduction in pigs.

Acknowledgements: This work was supported by the Irish Government under the National Development Plan 2000-2006.

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Survival of *Salmonella* serovar Typhimurium inside porcine monocytes is associated with complement binding and suppression of the production of reactive oxygen species

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Summary: Macrophages are thought to play a major role in the development of *Salmonella* carriers in swine. It was the aim of the present study to characterize the interactions of a *Salmonella* serovar

Typhimurium strain with porcine peripheral blood monocytes. The production of reactive oxygen species (ROS) by monocytes and the numbers of intracellularly killed bacteria differed significantly between the different pigs used. Opsonization of *Salmonella* bacteria with complement significantly decreased bacterial killing. Interestingly, monocytic ROS production was suppressed by metabolically active bacteria. In conclusion, binding to host complement and suppression of monocyte ROS production enable ser. Typhimurium to survive for at least 6 hours in porcine monocytes. Moreover, individual differences of porcine monocytes to produce ROS and to kill the intracellular *Salmonella* bacteria might account for the development of the carrier state in some pigs and not in others.

Keywords: *Salmonella* Typhimurium, pig monocyte, carrier, reactive oxygen species

Introduction: Persistent *Salmonella* infections in pigs result in contamination of carcasses in the slaughterhouse. The mechanism of this carrier state is poorly understood but is associated with survival of *Salmonella* bacteria inside host macrophages. However, studies on interactions of porcine mononuclear cells with *Salmonella* are scarce (Riber and Lind, 1999). It was therefore the aim of the present study to characterize the following interactions of *Salmonella* serovar Typhimurium with porcine monocytes: 1) production of reactive oxygen species 2) production of reactive nitrogen intermediates 3) the formation of spacious phagosomes (unusually wide, *Salmonella* containing endosomes) 4) bacterial killing and 5) cytotoxicity of the *Salmonella* bacteria on the porcine monocytes.

Materials and methods: Monocytes. Peripheral blood monocytes were collected from 14 to 24 week old pigs using density centrifugation on a ficoll-paque density gradient and subsequent adhesion to tissue culture flasks. Cell purity was determined using incubation with monoclonal mouse anti-SWC3 antibodies and flow cytometry. A purity of 85-90 % was obtained.
Salmonella strain. A serovar Typhimurium strain (20735c) isolated from pigs was used throughout the studies. Bacteria were grown for 6 h at 37 °C in Brain Heart Infusion, washed three times and resuspended in Hank's balanced salt solution (HBSS) at the desired concentration. Viable bacteria were opsonized with guinea pig complement (Virion ltd., Switzerland) during 30 min at 20 °C. Bacteria were inactivated using either UV light or acetone. In order to abolish protein synthesis, *Salmonella* bacteria were incubated with 25 _g/ml chloramphenicol.
Production of reactive oxygen species by porcine monocytes after stimulation with *Salmonella* Typhimurium. Production of reactive oxygen species (ROS) was determined using luminol-enhanced chemiluminescence (CL). Briefly, 10^6 monocytes were seeded per well containing 200 _M luminol and exposed either to viable, inactivated or chloramphenicol treated bacteria at 10 bacteria per monocyte or to phorbol myristate acetate (PMA) at 20 _g/ml. Viable bacteria were used either native or opsonized with guinea pig complement. CL was recorded during 1 h. At 1 h after stimulation with *Salmonella*, the residual monocyte activity was assessed by the addition of 20 _g/ml PMA and recording of CL for another hour. The test was performed on monocytes from 4 pigs and repeated three times in triplicate.
Production of reactive nitrogen intermediates by porcine monocytes after stimulation with *Salmonella* Typhimurium. Production of reactive nitrogen intermediates (RNI) was determined using the Griess reaction. Briefly, 10^6 monocytes were seeded per well and inoculated with 10^7 *Salmonella* bacteria. After centrifugation at 364 x g for 10 min at 37 °C and subsequent incubation for 30 min at 37 °C, the culture medium was replaced by medium containing 10 _g/ml gentamicin to kill extracellular bacteria. After 24 h of incubation at 37 °C, 100 _l of supernatant was collected per well and the Griess reaction was performed. The test was performed on monocytes from 4 pigs and repeated three times in triplicate.
Spacious phagosome formation in porcine monocytes after stimulation with *Salmonella* Typhimurium. The formation of spacious phagosomes (SP) was determined using a previously described technique (Alpuche Aranda et al., 1995). This technique is based on the fluorescent visualization of SP by inclusion of FITC-labelled dextran and subsequent microscopic evaluation. The test was performed on monocytes from 2 pigs.

Killing of *Salmonella* Typhimurium by porcine monocytes. Monocytes were seeded at 10^5 cells per well and exposed to 10^6 *Salmonella* bacteria as described in the RNI assay. The bacteria were used either native or opsonized with guinea pig complement. At 0, 2 and 6 hours after exposure, the cells were rinsed, lysed and the number of *Salmonella* bacteria was counted by plating tenfold serial dilutions on brilliant green agar. The test was performed on monocytes from 4 pigs and repeated at least three times in triplicate.
Cytotoxicity of *Salmonella* Typhimurium on porcine monocytes. Monocytes were inoculated with *Salmonella* as described in the CL assay. After 2 h of incubation, the supernatant was collected and the level of lactate dehydrogenase activity was measured (Roche Diagnostics GmbH). The test was performed on monocytes from 4 pigs and repeated three times in triplicate.

Results:

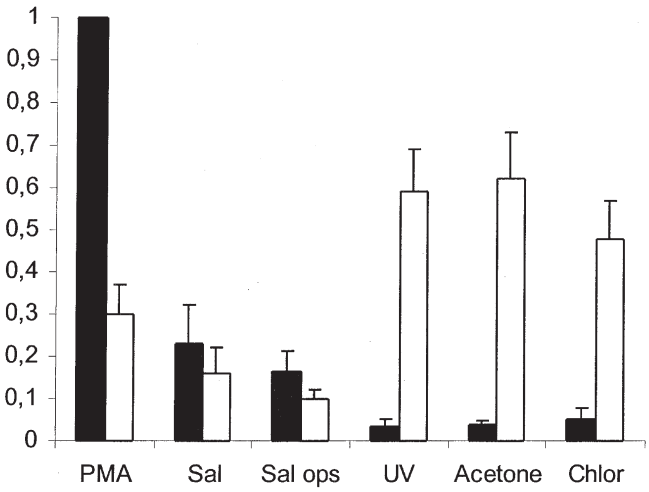

Fig. 1. *Production of reactive oxygen species by porcine monocytes. The black bars represent the average chemiluminescent responses ± s.e. of monocytes exposed to PMA, viable salmonellae (Sal), opsonized bacteria (Sal ops), UV or acetone inactivated bacteria or chloramphenicol treated salmonellae (chlor). Values are expressed as fractions of the peak value obtained after stimulation with PMA. White bars represent the average residual activity ± s.e.*

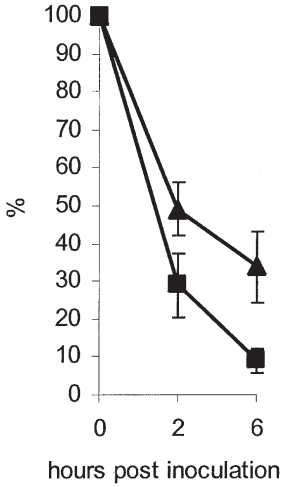

Fig. 2. *Average percentage ± s.e. of intracellular survival of Salmonella bacteria in porcine monocytes. Bacteria were either native (squares) or opsonized (triangles).*

Production of ROS and RNI and the formation of SP by porcine monocytes exposed to *Salmonella* Typhimurium. The results of the CL assays are shown in Figure 1. Viable salmonellae induced a higher CL response compared to inactivated or chloramphenicol treated bacteria. Contrary to exposure to inactivated and chloramphenicol treated bacteria, monocytes exposed to viable bacteria showed only marginal residual activity. The monocytes from one pig produced approximately three times more ROS than those from the other three (one way ANOVA, $P < 0.05$). The *Salmonella* strain did not cause cytotoxicity in the monocytes at 2 h post inoculation. No detectable amounts of RNI were produced by the porcine monocytes in none of the test conditions. In 9-12 % of the *Salmonella* containing monocytes, spacious phagosomes were detected.
Killing of *Salmonella* Typhimurium by porcine monocytes. Results of the microbicidal assays are summarized in Figure 2. Numbers of intracellular salmonellae decreased significantly less (paired t-test, $P < 0.05$) when the bacteria were opsonized with guinea pig complement. Monocytes from one pig were significantly less capable of killing *Salmonella* bacteria between 0 and 2 h post inoculation compared to those from the other three pigs (approximately 2.5 times less; one way ANOVA, $P < 0.05$).

Discussion: Most of the pathogenesis of salmonellosis has been described in mice, chickens and calves. Few data exist on the interactions of *Salmonella* with porcine phagocytes. The lack of RNI production by the porcine monocytes demonstrates that not all data collected from mice can be extrapolated to other species. In mice, the production of NO by inducible NO synthase (iNOS) is important in controlling intracellular multiplication of *Salmonella* bacteria (Umezawa et al., 1997). In contrast with Riber and Lind (1999), the number of intracellular bacteria steadily decreased over the 6 h period, suggesting lack of intracellular bacterial multiplication. Interestingly, opsonization with complement increased the number of surviving salmonellae. Possibly, intracellular trafficking and thus survival of the *Salmonella* bacteria might be influenced by entry in the host cell through complement receptor binding (Ishibashi and Arai, 1996). The production of ROS by host macrophages is an important first defence mechanism (Vazquez Torres et al., 2000), which *Salmonella* must circumvent in order to survive intracellularly inside the host cell. The *Salmonella* Typhimurium strain was able to suppress the production of ROS in porcine monocytes. This suppression was abolished when chloramphenicol treated bacteria were used, indicating that suppression of monocytic ROS production requires active bacterial protein synthesis. Individual differences between pigs were noticed both in the production of ROS and in the ability to kill *Salmonella*. These individual differences might account for a different course of infection, for example the development of the carrier state in some but not in other pigs.

Acknowledgements: These studies were financially supported by FOD Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu, DG4, Brussels, Belgium

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O 42 The stomach acts as a barrier against *Salmonella* in pigs fed a meal diet

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Summary: Finishing pigs fed a coarsely ground meal (CGM) diet showed increased *in vitro* death rate of *Salmonella* in the gastric content and a reduced number of enterobacteria in the small intestine and caecum compared with a finely ground and pelleted diet (FGP). The CGM diet resulted moreover in a slower gastric emptying rate, increased the DM content and established a pH-gradient in the stomach. This affected the microbiota in the gastric digesta resulting in more lactic acid